AD-A102 982 SYSTEMS RESEARCH LABS INC DAYTON OHIO F/G 6/20
BLOOD CHOLINESTERASE AS A FUNCTION OF PHYSOSTIGMINE.(U)
JUL 81 M E CAMPBELL, D W BLICK, J LANUM F33615-80-C-0603
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Report SAM-TR- 81-19

LEVEL II

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BLOOD CHOLINESTERASE AS A FUNCTION OF PHYSOSTIGMINE

Michael E. Campbell, Ph.D.
Dennis W. Blick, Ph.D.
Jackie Lanum, Ph.D.
Thomas G. Wheeler, Ph.D.
Grant D. Callin, Major, USAF (USAFSAM/RZW)
Systems Research Laboratories, Incorporated
2800 Indian Ripple Road
Dayton, Ohio 45440

July 1981

Final Report for Period May 1980 - March 1981

Approved for public release; distribution unlimited.

Prepared for
USAF SCHOOL OF AEROSPACE MEDICINE
Aerospace Medical Division (AFSC)
Brooks Air Force Base, Texas 78235

NOTICES

This final report was submitted by Systems Research Laboratories, 2800 Indian Ripple Road, Dayton, Ohio 45440, under contract F33615-80-C-0603, job order 7757-05-43, with the USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, Brooks Air Force Base, Texas. Major Grant D. Callin was the Laboratory Project Scientist-in-Charge.

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The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act of 1970 and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources - National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

Grant D. Callin

GRANT D. CALLIN, Major, USAF
Project Scientist

Donald N. Farrer

DONALD N. FARRER, Ph.D.
Supervisor

Roy L. DeHart

ROY L. DEHART
Colonel, USAF, MC
Commander

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: SAM-TR-81-19	2. GOVT ACCESSION NO.: AD-A102982
3. RECIPIENT'S CATALOG NUMBER:	
4. TITLE (and Subtitle): BLOOD CHOLINESTERASE AS A FUNCTION OF PHYSOSTIGMINE	5. TYPE OF REPORT & PERIOD COVERED: Final Report, May 1980 – Mar 1981
	6. PERFORMING ORG. REPORT NUMBER:
7. AUTHOR(s): Michael E. Campbell, Dennis W. Blick, Jackie Lanum, Thomas G. Wheeler (Systems Research Laboratories); Grant D. Callin, Major, USAF (USAFSAM/RZW)	8. CONTRACT OR GRANT NUMBER(s): F33615-80-C-0603
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Systems Research Laboratories, Inc., 2800 Indian Ripple Road, Dayton, Ohio 45440	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: 62202F, 7757-05-43
11. CONTROLLING OFFICE NAME AND ADDRESS: USAF School of Aerospace Medicine (RZW), Aerospace Medical Division (AFSC), Brooks Air Force Base, Texas 78235	12. REPORT DATE: Jul 1981
	13. NUMBER OF PAGES: 5
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	15. SECURITY CLASS. (of this report): UNCLASSIFIED
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

blood cholinesterase
physostigmine

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The purpose of this research was to document, by the pH method, changes in blood cholinesterase after carbamate poisoning. Five doses of physostigmine and a placebo were injected into rhesus monkeys, and blood samples were taken periodically for 48 hours. Cholinesterase inhibition and behavioral symptoms were most severe during the first 4 hours post injection, and recovery was essentially complete by 8 hours. The results will be used to determine dose and temporal parameters in future studies with physostigmine.

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BLOOD CHOLINESTERASE AS A FUNCTION OF PHYSOSTIGMINE

Inhibition of cholinesterase (ChE), the enzymes normally responsible for acetylcholine (ACh) lysis, results in neurotoxicity due to increased ACh concentrations throughout the nervous system. ChE is found in most tissues of mammals. The blood is readily sampled and assayed for ChE, and most behavioral effects originate in the brain.

Stitcher et al. (6) have demonstrated the relationship between changes in blood ChE levels and brain ACh levels in rats as the result of soman poisoning. As blood and brain ChE activity was reduced to below 15% normal, brain ACh levels increased to nearly 140% of control values. Hollt et al. (1) showed that the binding properties of various organophosphates to serum cholinesterase and brain homogenate are similar, indicating that the enzymes are the same in blood and brain, although there is evidence of differential origins of ChE in these organs (5). Thus, ChE is functionally similar in blood and brain and is similarly affected by organophosphate insult to the organism.

Little research is available on the time course of cholinesterase inactivation and/or reactivation after anticholinesterase compounds are administered in rhesus monkeys. Holmstedt (2) has pointed out the value of measuring blood cholinesterase for (1) the diagnosis of intoxication, (2) studies of efficient antidotes, and (3) determination of performance decrement and recovery.

Inferences regarding cholinesterase inhibition and behavior symptoms must be backed by adequate data on the time course of inhibition and recovery from anticholinesterase poisoning in primates. The dose-response relationships between various anticholinesterase drugs and blood cholinesterase levels have not been widely studied. Thus, acquiring this information prior to behavioral or histochemical studies of drug action in the rhesus monkey is important.

Reiter et al. (4) have provided limited parametric data for parathion in red blood cells and plasma of monkeys. Their results indicate maximum enzyme inhibition at 6 hours after oral administration of the drug. Recovery is virtually complete after 2 weeks. Behavioral deficits (on an appetitive discrimination task) occurred from 1 to 24 hours after drug administration. Similar data are not available for anticholinesterase drugs, which are of more general interest.

The present study examines the effects of physostigmine on blood cholinesterase levels and behavior in rhesus monkeys.

PROCEDURES

Subjects for this experiment were 13 male rhesus monkeys (Macaca mulatta), age 8-15 years, used in previous research on laser and particle beam effects on visual perception. Blood samples (.5 ml) were collected by venipuncture at 24, 22, and 20 hours prior to drug administration; at the time of drug administration; and at .5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 8, 12, 24, 36, and 48 hours after drug administration. Nine animals received a .05, .07, .09, .11, or .13 mg/kg dose of physostigmine salicylate or a placebo injection on two occasions, and four animals were dosed three times. Drug administrations for a given animal were at least 3 weeks apart. Order of drug-dose presentation was randomized for each animal. Blood assay procedures on red blood cells were conducted according to procedures described by Michael (3). This method measures red blood cell pH as a function of cholinesterase inhibition. Two control measures were incorporated in this design: (1) the blood samples taken prior to drug administration, and (2) placebo controls. The sequential sampling allowed for changes in blood cholinesterase levels over time to be determined as a function of drug and dose. Stress effects of procedures were controlled by the predrug baseline samples. Order effects (practice, habituation to procedure) were controlled by randomizing the order of drug-dose and placebo presentation. Animals remained unrestrained in home cages throughout the procedure and were closely observed during the first 3 hours post injection for clinical signs and behavioral changes.

RESULTS

A baseline value of red blood cell pH was determined for each animal by computing the mean pH of the first three(nondrug) blood samples. Then, changes from baseline for the different dose groups (including controls) were compared at each of the sampling times. Results of the analysis of pH change at each sampling time are presented in Table 1. The values in the far-right column are probabilities associated with the null hypothesis (i.e., that differences between groups are due to chance). Thus, these values may be used to gauge the time course of drug effects. Drug effects are seen to be largely dissipated after 8 hours, although they approach significance again at 36 hours. Figure 1 illustrates the changes in blood ChE over time after physostigmine administration. The greatest inhibition of blood cholinesterase is seen to occur at 1.5 to 2 hours after physostigmine administration, and recovery is complete by 8 hours. The variation in the data makes the degree of dose-dependency impossible to ascertain; however, the pattern of inhibition and recovery is the same for all doses except the placebo.

TABLE 1. SIGNIFICANCE TESTS OF RBC PH DIFFERENCE AMONG GROUPS AT VARIOUS TIMES AFTER DRUG ADMINISTRATION

Sampling time after drug administration (hr)	F(df)	P
.5	11.76(5,9)	.002
1.0	11.24(5,9)	.001
1.5	14.44(5,9)	<.001
2.0	12.47(5,9)	<.001
2.5	5.74(5,9)	.012
3.0	9.71(5,8)	.003
3.5	4.21(5,9)	.029
4.0	8.03(5,4)	.033
8.0	3.44(5,9)	.052
12.0	1.96(5,5)	.238
24.0	.40(5,5)	.832
36.0	4.84(5,5)	.054
48.0	3.11(5,5)	.119

Reliable behavior changes included lying down in the cage; lacrimation; muscle tremors about the face, neck, shoulders, arms, and hands; and aperiodic contraction of the cremaster. These behaviors were observed in animals receiving .09 mg/kg or more physostigmine, but never in animals receiving less. Also observed at the higher doses, but less reliably, were a generalized restlessness, huddling, and more frequent threat behaviors. (Presence or absence of these behaviors was recorded by observers who did not know what drug or dose, if any, the animal received.)

DISCUSSION

The present study indicates maximum inhibition of red blood cell cholinesterase at .5 to 4 hours after administration of .13 mg/kg physostigmine, with virtually complete recovery by 8 hours. Lower doses also produced lowered ChE values, but not in a dose-dependent manner and not markedly lower than placebo values during the same time period.

Behavioral effects were transient and occurred at lower dose levels than those producing significant blood ChE inhibition. This is important because blood ChE is to be used to predict performance after administration of anticholinesterase chemicals. Behavioral disruption occurs at lower levels of ChE inhibition than can be readily measured by the pH technique.

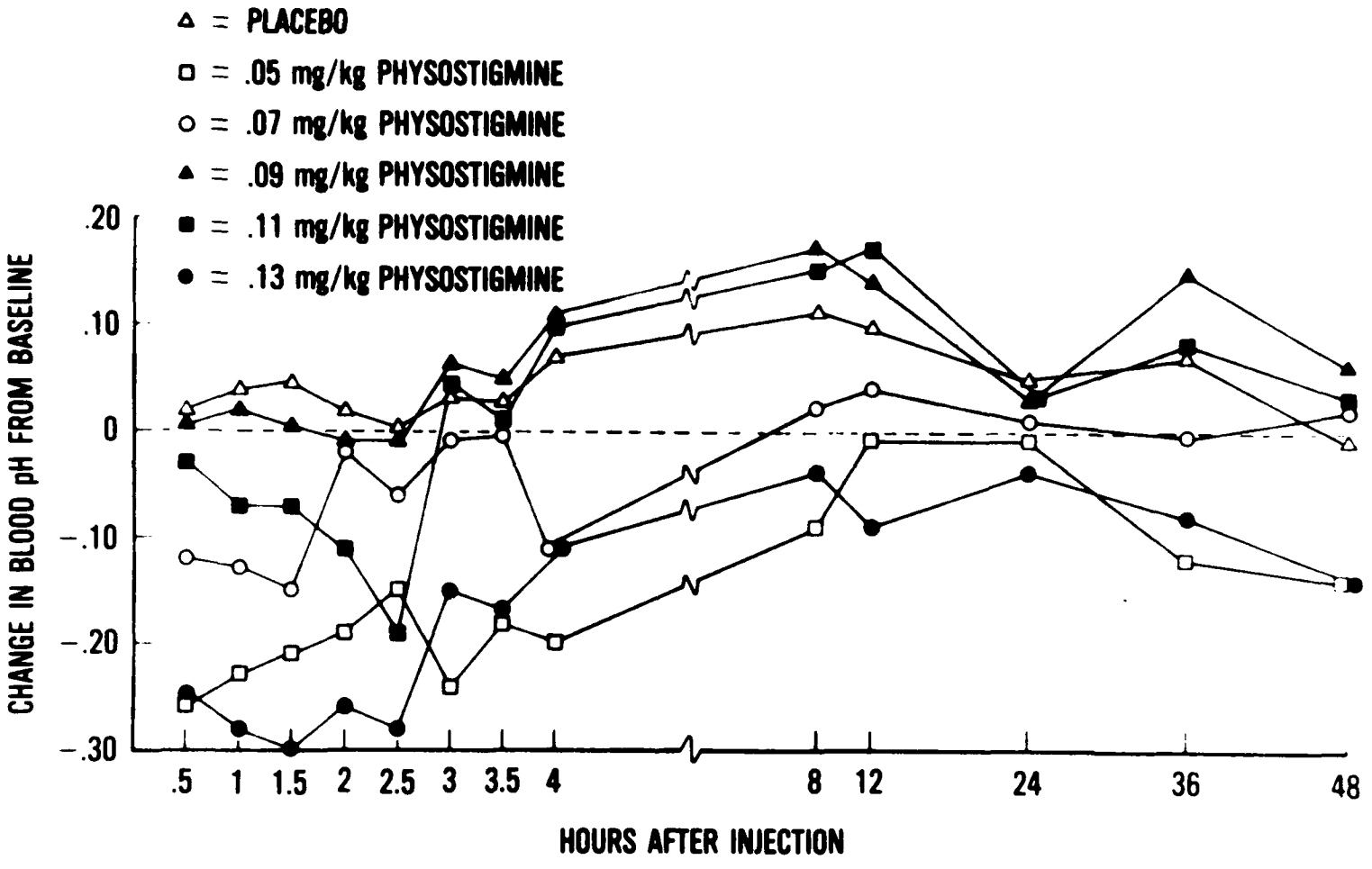

Figure 1. Change in red blood cell cholinesterase as measured by the pH method and as a function of time after an injection of physostigmine (mg/kg) or placebo.

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